PLGAs bearing carboxylated side chains: Novel matrix formers with improved properties for controlled drug delivery


Abstract

Novel PLGA derivatives bearing carboxylated side chains have been synthesized and used to encapsulate the fragile drug apomorphine HCl with a solid-in-oil-in-water solvent extraction/evaporation method. Blends of \( d,l \)-lactide and \( l-3-(2\text{-}\text{Benzyloxy}c\text{arbonyl})\text{Ethyl-1,4-Dioxane-2,5-dione (BED)} \) were co-polymerized at different ratios via ring-opening using benzyl alcohol as initiator. Optionally, the ester groups in the side chains as well as the terminal ester groups were hydrolyzed (leading to free –COOH groups). For reasons of comparison, different types of conventional PLGAs were also synthesized and used for apomorphine HCl encapsulation. The polymers and microparticles were thoroughly characterized using SEC, \(^1\)H NMR, DSC, SEM, X-ray and laser diffraction, Headspace-GC as well as in vitro drug release measurements in flow-through cells and agitated flasks. Importantly, microparticles based on the new polymers bearing carboxylic groups in the polymeric side chains: (i) allowed a significant reduction of the amount of residual solvent (dichloromethane), and (ii) provided different types of drug release patterns compared to microparticles based on "conventional" PLGAs (at least partially due to altered polymer degradation kinetics). Thus, they offer an interesting potential as novel matrix formers in controlled drug delivery systems, overcoming potential shortcomings of standard PLGAs.

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1. Introduction

Poly(lactic-co-glycolic acid) (PLGA) copolymers have been widely used for biomedical and pharmaceutical applications, ranging from tissue engineering to drug delivery, due to their biodegradability and good biocompatibility [1,2]. Nowadays, they are often the choice as matrix formers for parenteral controlled drug delivery systems, and several products are available on the market. PLGA based microparticles are known to undergo in-situ forming and pre-formed implants [3] as well as microparticles [4] can be used to control the release of low molecular weight drugs [5] and of therapeutically active macromolecules, e.g. proteins [6]. Varying the lactic acid:glycolic acid ratio and average polymer molecular weight, drug release periods ranging from a few days up to several months can be achieved [7–9]. The underlying mass transport mechanisms controlling drug release can be highly complex, involving water and drug diffusion, polymer degradation, matrix erosion and eventually limited solubility as well as autocatalytic effects [10–15]. Upon contact with aqueous media, water penetrates into the systems and the polymers degrade via hydrolysis into shorter acid chains. PLGA based microparticles are known to undergo "bulk erosion", since the penetration of water into the system is faster than the subsequent polymer chain cleavage [16]. The degradation rate is affected by the lactic acid:glycolic acid ratio [17]. Furthermore, the rate and extent of water uptake greatly depends on the polymer characteristics, in particular the latter's hydrophilicity and crystallinity.

The introduction of functional groups in side chains along the polymer backbone can be used to alter key properties of these macromolecules. Several PLGA derivatives have been proposed in the past [e.g., 18–24]. An excellent overview on the synthesis and biomedical/pharmaceutical applications of functional aliphatic polyesters has been recently given by Seyednejad et al. [25]. In principle, two different synthesis strategies can be distinguished: 1) the functionalization of pre-formed PLGA, and 2) the polymerization of functionalized monomers/dimers. In practice, often the second option is preferred.
due to the risk of side reactions (e.g. chain scission and racemization) associated with the first strategy. The polymerization of functionalized mono/dimers can generally be conducted via: (i) polycondensation, (ii) ring-opening polymerization (ROP), or (iii) enzymatic polymerization. ROP is often preferred, since high molecular weight polymers can be obtained relatively easily in a controlled manner (in contrast to the other two options). A variety of functionalized PLGAs has been proposed, bearing different types of side chains and end groups [21,22,26]. A particularly interesting type of polymers has been proposed by Hennink and co-workers: poly(lactic-co-hydroxymethyl glycolic acid)s (PLHMGAs) [27–30]. These PLGAs bear side chains with free \(-\text{OH}\) groups, rendering the polymers more hydrophilic than “conventional” PLGAs. Thus, the rate and extent of water uptake can be increased and properties, such as cell adhesion improved. Also, ester hydrolysis can be accelerated [27] and the in vitro degradation rate increased [28]. Importantly, PLHMGAs can also reduce the risk of chemical modifications of incorporated peptide drugs when used as matrix formers, compared to “conventional” PLGAs [31]. Furthermore, they have been shown to be highly suitable for the encapsulation of very different types of drugs/model compounds, including gentamicin, BSA, dextran blue and lysozyme [29,30,32]. However, there is still a need for further improvements of key properties of biodegradable and biocompatible matrix formers in controlled drug delivery systems [25,33,34].

The aim of the present study was to synthesize new PLGA derivatives, bearing carboxylated side chains, in order to improve the polymer characteristics. A ring-opening polymerization of different blends of \(\text{D,L-lactide and glycolide}\) (PLGA), and \(\text{D,L-lactide and L-3-(2-Benzyloxycarbonyl)Ethyl-1,4-Dioxane-2,5-dione (BED)}\) was conducted in solution using benzyl alcohol as initiator. Optionally, the terminal benzyl ester groups as well as the benzyl ester groups in the side chains were hydrogenolyzed, leading to free \(-\text{COOH}\) groups. These novel polymers were used to encapsulate the fragile drug apomorphine HCl with a solid-in-oil-in-water solvent extraction/evaporation method. The obtained microparticles were thoroughly characterized in vitro, in particular with respect to their drug release behavior. For reasons of comparison, different types of “conventional” PLGAs were also synthesized and used for microparticle preparation.

2. Materials and methods

2.1. Materials

\(\text{D,L-lactide and glycolide}\) (Purac, Gorinchem, The Netherlands) were recrystallized in toluene. Mesitylene (99%; Acros Organics, Geel, Belgium) was dried over molecular sieves (4 Å, powder; Aldrich, Saint-Quentin-Fallavier, France) until residual amounts of water were below 5 ppm. Benzyl alcohol (99+%; Aldrich) was dried over sodium and distilled before use. Thiourea catalyst (TUcHex) was prepared according to Pratt et al. [35] and purified via triple recrystallization from chloroform. \((-\)\)-Sparteine (99%; Aldrich) was distilled twice over calcium dihydride and stored under argon. \(\text{L-3-(2-Benzoxycarbonyl)Ethyl-1,4-Dioxane-2,5-dione (BED)}\) was prepared according to Thillaye du Boullay et al. [21]. Tin octoate (Aldrich), poly(D,L lactic-co-glycolic acid) (PLGA; Resomer RG 502H; PLGA 50:50; Boehringer Ingelheim, Ingelheim, Germany), apomorphine hydrochloride (R-apomorphine HCl; Francopia, Paris, France), acetonitrile, dichloromethane (HPLC Grade; Fisher Scientific, Loughborough, UK), polyvinyl alcohol (Mowiol 4-88; Sigma-Aldrich, Steinheim, Germany), ascorbic acid (Cooper, Melun, France), Celite®545 (Aldrich, France) and ethylenediaminetetraacetic acid (EDTA; VWR, Haasrode, Belgium) were used as received.

Fig. 1. Schematic presentation of the synthesis of the investigated copolymers, based on: a) \(\text{D,L-lactide and glycolide}\) (PLGA), and b) \(\text{D,L-lactide and L-3-(2-Benzoxycarbonyl)Ethyl-1,4-Dioxane-2,5-dione (PLA-co-PBED)}\).
2.2. Polymer synthesis

2.2.1. PLGA with – COOH end groups and 50:50 monomer ratio (Fig. 1a)

Benzy1 alcohol (1 equiv.) was added to a 1 M mixture of glycolide (n equiv.) and γ-L-lactide (n equiv.) in mesitylene. The reaction mixture was heated to 165 °C. After complete dissolution, a 0.1 M solution of tin octanoate (0.1 equiv.) was added at once under rapid stirring. The reaction mixture was kept under a moderate reflux until completion. The reaction mixture was cooled down to room temperature and the solvent was eliminated by decantation (90% of the initial volume was recovered). The obtained polymer was dissolved in 20 volumes of dichloromethane and washed under stirring for 1 h with 0.5 N aqueous HCl (15 volumes), then twice with 10 volumes of water. The organic solution was dried over anhydrous sodium sulfate and the solvent eliminated under reduced pressure to yield “protected” PLGA (benzyl ester end-capped) as white-off foam.

To a solution of the protected PLGA in acetone (15 volumes) was added the catalyst (5% Pd on charcoal) and the reaction mixture was stirred under hydrogen atmosphere for 3 h. The catalyst was removed by filtration and the polymer was precipitated by addition of cold methanol (3 h) of the former solution onto 150 volumes of pentane under mechanical stirring. After decantation, the polymer was dissolved in dichloromethane (10 volumes) and dried under reduced pressure (24 h, 50 °C, 0.5 mbar) to yield the PLGA as a white foam. The global yield was around 90% over the two steps.

2.2.2. PLA-co-PBED n:m (copolymer with m mol% of pendant benzyl protected COOH) (Fig. 1b, top raw)

Benzy1 alcohol (1 equiv.) was added to a 1.2 M solution of γ-L-lactide (n equiv.) in dichloromethane. The reaction mixture was heated at 30 °C and a solution of the catalysts (3 equiv of Tl(nOct)2 and 1.5 equiv of sparteine) in dichloromethane (5 volumes) was added at once. After 30 s, a solution of L-3-(2-Benzyloxycarbonyl) Ethyl-1,4-Dioxane-2,5-dione (BED) (m equiv.) in dichloromethane (10 volumes) was added dropwise (12 min). After complete conversion, sparteine was neutralized by addition of 10 equiv. of benzoic acid and the reaction mixture was concentrated to half of the initial volume and was poured over cold methanol (40 volumes) and stirred for 1 h. Methanol was eliminated and the polymer was washed with cold methanol (8 volumes) to yield a sticky solid. A white solid (copolymer with ester end groups and ester side chains) was obtained after trituration with cold diethyl ether.

2.2.3. PLA-co-PBED n:mH (copolymer with m mol% of pendant free – COOH, and with free – COOH end groups) (Fig. 1b bottom raw)

The removal of the benzyl groups was achieved by hydrogenolysis of the protected copolymer in acetone (15 volumes) with 10% palladium on charcoal as catalyst under hydrogen atmosphere. After total deprotection (confirmed by the complete disappearance of the aromatic signals on the 1H NMR spectra), the catalyst was removed by filtration over Celite® and the solvent was distilled under reduced pressure to yield a copolymer white foam.

2.3. Polymer characterization

NMR Spectra were recorded in CDCl3 or acetone d6 on a Bruker Avance 300 MHz spectrometer (Bruker, Hanau, Germany) at room temperature. Chemical shifts are reported in ppm relative to Me4Si as an external standard. The number-average and weight-average molar masses (Mn and Mw, respectively) and molar mass distributions (Mw/Mn) of the polyesters (bulk materials and microparticles) were determined by SEC at 40 °C with a Waters 600 liquid chromatography, equipped with a Waters 2410 Refractive Index Detector and are not corrected (elucent = tetrahydrofuran, flow rate = 1 mL/min, Waters pre-column, Waters STYRAGEL column: HR 4E, 50–100,000 g/mol) (Waters, Guyancourt, France). Calibrations were performed using polystyrene standards (400–100,000 g/mol).

2.4. Microparticle preparation

Apomorphine HCl-loaded microparticles were prepared using a solid-in-oil-in-water (S/O/W) solvent extraction/evaporation technique as follows: 320/360 mg polymer and 80/40 mg drug (20/10% theoretical drug loading) were dissolved/dispersed within 3 mL dichloromethane by sonication (Sonopuls UW2070, 30% amplitude, 2 min; Bandelin, Berlin, Germany). This dispersion was injected using a syringe and 20 Gauge-needle into 50 mL of an outer aqueous polyvinyl alcohol solution (5% w/w; 4 °C; protected from light) under stirring with a three-blade propeller (700 rpm). Stirring was continued for 20 min. The obtained particles were hardened by adding 200 mL water (4 °C) and further stirring (700 rpm, 3 min). To promote extraction of dichloromethane, the suspension was poured into 600 mL water (4 °C; protected from light) and stirred for 40 min (700 rpm). The particles were separated by filtration using a nitrocellulose membrane (3 μm), rinsed with water and subsequently dispersed in water. The suspension was frozen at −45 °C for 2 h and freeze-dried to minimize residual solvents’ content (primary drying: 0.014 mbar, −9 °C shelf temperature, 10 h; secondary drying: 0.0014 mbar, 20 °C shelf temperature, 10 h) (Epsilon 2 LSC; Christ, Osterode, Germany). Microparticles were stored under nitrogen at 4 °C and protected from light. Three batches of microparticles were prepared for each formulation.

2.5. Differential scanning calorimetry (DSC)

Thermograms of polymers and micro particles were recorded with a DSC1 Star System (Mettler Toledo, Greifensee, Switzerland). Approximately 5 mg samples were heated in sealed aluminum pans from −10 °C to 100 °C at 10 °C/min, hold for 5 min, cooled at 10 °C/min to −10 °C and reheated from −10 °C to 100 °C at 10 °C/min. Glass transition temperatures (Tgs) were determined from the second heating cycles.

Table 1

<table>
<thead>
<tr>
<th>Polymer</th>
<th>% Glycolide</th>
<th>% γ-L-lactide</th>
<th>BED</th>
<th>Mw, g/mol</th>
<th>Mn, g/mol</th>
<th>Mw/Mn</th>
<th>Tg, °C (onset)</th>
<th>Physical state</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA 50:2H</td>
<td>50</td>
<td>50</td>
<td>–</td>
<td>10,320</td>
<td>5270</td>
<td>1.96</td>
<td>42</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLGA 50:4H</td>
<td>50</td>
<td>50</td>
<td>–</td>
<td>4440</td>
<td>1960</td>
<td>2.26</td>
<td>31</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLGA 50:10K</td>
<td>50</td>
<td>50</td>
<td>–</td>
<td>9500</td>
<td>4620</td>
<td>2.05</td>
<td>38</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLGA 50:19K</td>
<td>50</td>
<td>50</td>
<td>–</td>
<td>18,790</td>
<td>8580</td>
<td>2.19</td>
<td>43</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLA-co-PBED 92:8H</td>
<td>–</td>
<td>92.5</td>
<td>7.5</td>
<td>13,336</td>
<td>12,008</td>
<td>1.11</td>
<td>45</td>
<td>Amorphous</td>
</tr>
<tr>
<td>PLA-co-PBED 98:2</td>
<td>–</td>
<td>98.4</td>
<td>1.6</td>
<td>18,744</td>
<td>17,124</td>
<td>1.10</td>
<td>54</td>
<td>Semi-crystalline</td>
</tr>
<tr>
<td>PLA-co-PBED 98:2H</td>
<td>–</td>
<td>98.3</td>
<td>1.7</td>
<td>19,020</td>
<td>17,255</td>
<td>1.10</td>
<td>39</td>
<td>Amorphous</td>
</tr>
</tbody>
</table>

* Mole% determined by 1H NMR.
Fig. 2. $^1$H NMR spectra of the investigated copolymers.
2.6. X-ray diffraction

X-ray powder diffraction analysis was performed with a Panalytical X’pert Pro diffractometer (λ Cu K α = 1.54 Å) in Bragg–Brentano 0–0 geometry (PANalytical, Almelo, The Netherlands). The powder samples were placed in a spinning flat sample holder.

2.7. Scanning electron microscopy (SEM)

The internal and external morphologies of the microparticles were studied using SEM (S-4700 Field Emission Gun; Hitachi, Hitachi High-Technologies Europe, Krefeld, Germany). Samples were covered under vacuum with a carbon layer. Cross-sections were obtained after inclusion into water-based glue (UHU, Buehl, Germany) and cutting with a razor blade.

2.8. Particle size analysis

Volume mean diameters and particle size distributions [expressed as span: (Dv, 90 – Dv, 10)/volume median diameter] were measured by laser diffraction (Mastersizer S; Malvern, Orsay, France). The microparticles were dispersed in water.

2.9. Determination of the practical drug loading

Approximately 10 mg of apomorphine HCl-loaded microparticles were placed into 1.5 mL of dichloromethane to dissolve the polymer in a glass tube. Then, 1.5 mL water (containing 0.2% ascorbic acid to avoid apomorphine HCl oxidation) was added. The tube was sonicated in an ultrasonic bath for 5 min to promote apomorphine transfer from the organic to the aqueous phase. Upon centrifugation at 3000 rpm for 3 min (Centrifuge Universal 320; Hettich, Tuttlingen, Germany) the two phases were separated and the water phase was removed. This extraction procedure was conducted 3 times. The drug content in the aqueous phase was determined by HPLC analysis (Varian ProStar 230 pump, 410 autosampler, 325 UV–vis detector, Galaxie software; Les Ulis, France). A reversed phase column C18 (Gemini 5 μm; 110 Å; 150 × 4.6 mm; Phenomenex, Le Pecq, France) was used. The mobile phase was acetonitrile:phosphate buffer pH 3 (75 mM) containing ethylenediaminetetraacetic acid (EDTA; 1 mM) (15:85 v/v), as described by Ng Ying Kin et al. [36]. The detection wavelength was 273 nm, the flow rate 1 mL/min. Fifty μL samples were injected. The elution time was around 8 min. Each experiment was conducted in triplicate.

Fig. 3. SEC chromatograms of the investigated copolymers.

Fig. 4. X-ray diffraction patterns of the investigated: a) copolymers (bulk powders), and b) apomorphine HCl-loaded microparticles (and apomorphine HCl bulk powder for reasons of comparison).

2.10. In vitro release studies

2.10.1. Agitated flasks

Microparticles (approximately 15 mg) were placed in 50 mL degassed phosphate buffer pH 7.4 (USP 35) containing 0.2% ascorbic acid.
acid (the latter was added to limit drug degradation in the bulk fluid) in glass flasks. The latter were closed under nitrogen, protected from light and horizontally shaken at 37 °C (80 rpm; GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At pre-determined time points, 3 ml samples were withdrawn (replaced with fresh medium) and analyzed by HPLC (as described above). Each experiment was conducted in triplicate.

10.2. Flow-through cells
Microparticles (approximately 15 mg) were incubated in a continuous flow-through cell, as described in detail by Aubert-Poussel et al. [37]. Briefly, the microparticles were placed into empty HPLC columns (4.6 mm × 5 cm; Omega; Upchurch Scientific, Oak Harbor, WA, USA), which were kept at 37 °C in a water bath. Degas phosphated buffer pH 7.4 (USP 35) containing 0.2% ascorbic acid was pumped through the columns at 2 ml/day (syringe pump PHD 2000; Harvard Apparatus, Les Ulis, France). The eluent was cooled at 4 °C and analyzed by HPLC as described above. Each experiment was conducted in triplicate.

2.11. Determination of residual solvents’ contents
Samples were analyzed using a Perkin Elmer Clarus 500 Gas Chromatograph, Clarus 560 Mass Spectrometer, Turbomatrix 16 HeadSpace sampler and Forte (30 m × 0.32 mm ID) BPX volatile columns (Perkin Elmer, Courtaboeuf, France). Polymers or microparticles (approximately 100 mg) were dissolved in 2 ml of dimethyl sulfoxide.

3. Results and discussion

3.1. Key properties of the investigated polymers
Different types of copolymers were synthesized from 50:50 blends of D,L-lactic acid and glycolic acid (“PLGA 50”) via the ring-opening polymerization illustrated Fig. 1a, followed by hydrogenolysis of the terminal benzyl esters. The end groups of the obtained polymers is indicated by an "OH and COOH groups on the other hand side. The presence of free COOH groups. In addition, a new type of PLGA derivatives was synthesized: when the molar mass was 10 kDa, the terminal groups were as well COOH groups as well as the side chains are benzyl esters (Fig. 1b, top raw).

The nomenclature indicates weight-average molar masses of the latter was added to limit drug degradation in the bulk fluid) in glass flasks. The latter were closed under nitrogen, protected from light and horizontally shaken at 37 °C (80 rpm; GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At pre-determined time points, 3 ml samples were withdrawn (replaced with fresh medium) and analyzed by HPLC (as described above). Each experiment was conducted in triplicate.

The number-average and weight-average molar masses (Mn and Mw) and molar mass distributions (Mw/Mn) of the polymers were determined by size exclusion chromatography (SEC) (Fig. 3). Importantly, the polydispersity indexes were similar for all 50:50 PLGA polymers and very low in the case of all PLA-co-PBED polymers. The glass transition temperatures (Tgs) were measured by differential scanning calorimetry (DSC). For the 50:50 PLGAs the Tg was in the range of 31–43 °C and increased with increasing molecular weight. Interestingly, PLA-co-PBED 92:8H showed a higher Tg than PLA-co-PBED 98:2H (45 versus. 39 °C). This might be explained by more important hydrogen bonding due to the higher number of –COOH groups [38]. The physical state of the different polymer bulk powders was analyzed by X-ray diffraction. As it can be seen in Fig. 4a, all PLGA types and all PLA-co-PBED types bearing free –COOH groups seemed to be amorphous, whereas PLA-co-PBED 98:2 was semi-crystalline.

3.2. Key properties of apomorphine HCl-loaded microparticles
Apomorphine HCl-loaded microparticles based on the different copolymers were prepared using a solid-in-oil-in-water (S/O/W) solvent extraction/evaporation method. The theoretical drug loading was 20%. In all cases, spherical particles were obtained, the key properties of which are shown in Table 2. The mean diameter of the particles, measured by laser diffraction, ranged from 28 to 38 μm, as a function of the polymer used. In the case of the synthesized 50:50 PLGAs with free –COOH end groups, the practical drug loading was around 6% (corresponding to an encapsulation efficiency of around 30%), irrespective of the polymer molecular weight. The practical drug loading almost doubled (11%, corresponding to an encapsulation efficiency of 54%), when the commercially available RG 502H was used. An increase in encapsulation efficiency was also observed for the new PLA-co-PBED esters after hydrogenolysis of the benzyl esters: 5 versus 11% apomorphine HCl were encapsulated in the case of PLA-co-PBED 98:2 versus PLA-co-PBED 98:2H (corresponding to an increase of the encapsulation efficiency from 26 to 53%). This steep increase in encapsulation efficiency might at least partially be attributed to accelerated polymer precipitation during microparticle preparation: The presence of –COOH end groups (instead of benzyl ester end groups) renders the system more hydrophilic, resulting in more rapid water penetration into the inner phase. More rapid microparticle formation is likely to result in reduced drug loss into the outer aqueous phase. This hypothesis is consistent with the increased porosity of the microparticles, as observed by scanning electron microscopy (SEM) (Fig. 5). Interestingly, the glass transition temperatures of the investigated polymers were higher in
the microparticles compared to the initial bulk powders (Table 2 versus 1), except for PLA-co-PBED 98:2.

When preparing microparticles using an organic solvent, a particularly crucial system property is the residual solvents' content. Table 2 shows that remaining dichloromethane concentrations within the microparticles under identical manufacturing conditions (including those for freeze drying). Dichloromethane is toxic and its concentration in the final drug product must be reduced below the authorized limit (e.g., 600 ppm) [39]. Importantly, the residual solvents' contents were much lower in the case of the newly proposed PLGA derivatives compared to frequently used 50:50 PLGAs (including the commercially available PLGA 502H) (note that no efforts have been undertaken to further reduce the reported residual solvents' contents by optimized freeze-drying conditions). In the case of PLA-co-PBED 92:8H and PLA-co-PBED 98:2H, the dichloromethane concentrations were below 5 ppm. This is a major practical advantage of the novel copolymers: They allow for facilitated elimination of toxic dichloromethane residues. This can probably

Fig. 5. Effects of the type of polymer on the morphology of apomorphine HCl-loaded microparticles observed by SEM before exposure to release medium.
be attributed to a higher permeability of these polymers for this solvent. The fact that PLA-co-PBED 98:2 showed higher residual solvents’ contents might at least partially be explained by its semi-crystalline nature (reducing the permeability for dichloromethane). The microparticles’ contents in residual pentane and mesitylene (remaining from the polymer synthesis) are given in Table 2.

To characterize the physical state of apomorphine HCl within the polymeric matrices, the systems were analyzed by X-ray diffraction. As shown in Fig. 4b, the drug bulk powder was crystalline and at least partially remained in this state in microparticles based on PLGA 502H, PLGA 50-19kH and all PLA-co-PBED polymers. In contrast, the diffraction peaks were less visible in the case of PLGA 50-10kH-based microparticles and the drug was either in the amorphous state and/or molecularly dispersed within the polymeric matrix. In the case of PLGA 50-4kH, no diffraction peaks were visible.

### 3.3. In vitro drug release

The release of intact apomorphine HCl from microparticles based on 50:50 PLGAs with different molecular weights in phosphate buffer pH 7.4 (containing 0.2% ascorbic acid) in agitated flasks is shown in Fig. 6a. Clearly, the relative drug release rate significantly increased with decreasing polymer molecular weight. This can at least partially be attributed to the increased macromolecular mobility (as also evidenced by a decreased glass transition temperature, Table 1) and, thus, increased drug mobility within the microparticles [40]. Note that upon exposure to the release medium, water penetrates into the systems, leading to a significant decrease in the Tg of the polymer [41]. If the glass transition temperature is below 37 °C (temperature of the release medium), the polymer is in the rubbery state, allowing drug molecules to diffuse within the polymeric networks at much higher rates than in the glassy state. However, also other factors are likely to play a role: As it can be seen in Figs. 5 and 7, microparticles based on PLGA 50-4kH were much less porous than all other types of systems, before exposure to the release medium as well as after 7 days exposure to phosphate buffer pH 7.4. This might be due to a lower polymer precipitation rate during microparticle manufacturing and should be expected to result in slower drug release. However, as shown in Fig. 6a, apomorphine HCl release was much faster than for the other 50:50 PLGA types. This can be explained by the smaller particle size (Table 2), resulting in reduced diffusion pathway length and, hence, accelerated drug release. The smaller microparticle size can be attributed to the lower viscosity of the inner organic phase, leading to smaller droplets at similar shear forces.

Interestingly, apomorphine HCl release was rather similar in the case of microparticles prepared with the commercially available PLGA 502H and the synthesized PLGA 50-10kH. The observed difference in the initial release rate (“burst effect”) can probably be attributed to the difference in the initial drug loading (Table 2). Often, the burst release is caused by the presence of drug close to the microparticles’ surface [42,43]. Several studies reported an impact of the initial drug loading on this burst release [44,45]. To evaluate the validity of this hypothesis, PLGA 502H-based microparticles with a lower initial drug loading were prepared (theoretical loading = 10%), keeping all processing parameters constant. The mean diameter of the obtained microparticles was 35 μm (Span 1.3), the percent yield 93 (±5.3)% and the practical drug loading 5.8 (±0.3)% [corresponding to an encapsulation efficiency 59 (±3)%], thus, similar to the investigated PLGA 50-10kH based microparticles. The increase in yield and encapsulation efficiency might at least partially be attributable to the higher viscosity of the inner organic phase (the total amount of “polymer plus drug” was kept constant). Importantly, the burst release of apomorphine HCl indeed significantly decreased with decreasing practical drug loading: 39 and 12% drug were released after 1 h exposure to phosphate buffer pH 7.4 in agitated flasks. This confirms the above stated hypothesis: “The higher burst release of PLGA 502H microparticles compared to PLGA 50-10kH microparticles can (at least partially) be attributed to the higher drug loading.”

Note that apomorphine HCl release leveled off well below 100% in all cases in Fig. 6a. Analysis of the remnants revealed that no intact drug remained within the microparticles at the end of the observation period. Thus, apomorphine HCl degradation within the microparticles and/or within the release medium was of significant importance in these cases. Apomorphine is known to be degraded in aqueous solution, even in the presence of antioxidants [36,46]. If degradation occurred only after drug release, this is unlikely to be a limitation in vivo: Once released the drug will be metabolized/eliminated by the living body in any way. A drug delivery system with time-controlled release cannot protect a drug after its release. Thus, the cumulative relative (100% = initial drug loading) release profiles of intact apomorphine HCl shown in Fig. 6a result from the superposition of two processes: (i) release of intact drug from the microparticles, and (ii) apomorphine HCl degradation within the microparticles and/or the bulk fluid. To avoid potentially misleading conclusions from these curves, the respective results are plotted in a different way in Fig. 6b: The experimentally measured concentrations of intact drug in the release medium at the sampling time points are shown. As it can be seen, the apomorphine HCl concentration increased with time, due to continued drug release, but partially also decreased: This was the case when drug degradation in the release medium...
was more important than simultaneous release of intact drug from the microparticles. As it can be seen in Fig. 6b, this occurred at the end of the release periods and partially also at intermediate time points. Similar, tri-phasic drug release patterns from PLGA based microparticles have been reported in the literature [47,48]: An initial burst release is followed by a phase of slow drug release and a final rapid drug release phase (which leads to complete drug exhaust). The first phase is often attributed to the release of drug adsorbed

Fig. 7. Effects of the type of polymer on the morphology of apomorphine HCl-loaded microparticles observed by SEM after 7 days exposure to phosphate buffer pH 7.4 in agitated flasks.
onto the microparticles' surface and/or located in surface-near pores with direct access to the bulk fluid. The intermediate, much slower drug release phase might be caused by drug diffusion through the PLGA network, whereas the final rapid drug release might result from a breakdown of the polymeric structure (as soon as a critical, minimal polymer molecular weight is reached).

Fig. 8 shows the experimentally measured concentration of intact apomorphine HCl in the release medium when microparticles based on the novel PLA-co-PBED polymers were exposed to the bulk fluid. As it can be seen, the concentration initially increased and then decreased in all cases. This can be attributed to the fact that at early time points the release rate of intact drug is higher than the rate of drug degradation in the bulk fluid, whereas afterwards the degradation rate in the bulk fluid is higher than the release rate of intact drug. Again, drug degradation occurring only after drug release is not a limitation for the performance of the drug delivery system in vivo. This phenomenon might be considered as an artifact created under the given in vitro release conditions. Importantly, the type of PLA-co-PBED significantly affected the resulting concentrations of intact apomorphine HCl in the release medium (Fig. 8). The concentrations were highest in the case of PLA-co-PBED 92:8H, which might at least partially be attributable to the relatively high number of free -COOH groups, rendering this copolymer more hydrophilic than the others. Thus, the rate and extent of water penetration into the system are likely to be the highest. Consequently, the mobility of apomorphine HCl can be expected to be highest. The fact that the experimentally measured concentrations of intact apomorphine HCl in the bulk fluid were lowest in the case of PLA-co-PBED 98:2 based microparticles can at least partially be attributed to the following reasons: (i) The initial drug loading was lower (as discussed above) (Table 2). Thus, less drug can be released (also resulting in less pores created upon drug exhaust). (ii) The presence of benzyl esters instead of free – COOH groups renders the polymer less hydrophilic. Thus, water penetration into the system is reduced and drug mobility within the microparticles can be expected to be lower. (iii) In contrast to PLA-co-PBED 92:8H and PLA-co-PBED 98:2H, PLA-co-PBED 98:2 is semi-crystalline (Table 1), resulting in reduced permeability. This is consistent with the observed higher dichloromethane content of these microparticles (Table 2).

In order to minimize the above described “artificial” drug degradation within the bulk fluid, also another experimental set-up was used for drug release measurements, adapted from Aubert-Pouessel et al. [37]. The idea is to expose the microparticles to a continuous flow of “fresh” release medium. Samples are collected in a refrigerator at 4 °C. Since the microparticles are intended for parenteral application, the flow rate of the release medium was low (2 mL/day) and the volume of the bulk fluid the microparticles were exposed to was small (empty HPLC columns). Fig. 9 shows the experimentally measured cumulative relative (100% value = initial drug loading) release of intact apomorphine HCl from the investigated microparticles in phosphate buffer pH 7.4 in flow-through cells (2 mL/day) (b) is a zoom on early time points).
Interestingly, the novel PLA-co-PBED copolymers offer a large spectrum of release profiles: PLA-co-PBED 92:8H based microspheres exhibit similar drug release patterns as the investigated 50:50 PLGA s, whereas PLA-co-PBED 98:2H and PLA-co-PBED 98:2 based microspheres show significantly different release patterns. Note that 1 and 38% intact apomorphine HCI remained within the PLA-co-PBED 98:2H and PLA-co-PBED 98:2 based microspheres at the end of the observation period. Thus, in these cases (partial) drug degradation also occurred within the delivery systems. This is not too surprising since apomorphine HCI is a fragile drug, the temperature was 37 °C, the microspheres were exposed to an aqueous medium and the time periods were very long (up to 3 months). With more stable drugs, this behavior is not expected.

The significantly slower drug release kinetics from microspheres based on PLA-co-PBED 98:2H and PLA-co-PBED 98:2 can at least partially be explained by the much slower degradation of these co-polymers upon exposure to the release medium. Table 3 lists the experimentally measured Mw, Mn and polydispersity index (PDI = Mw/Mn) values of the systems at t = 0 (before exposure to the release medium) and after 13 days microsphere exposure to phosphate buffer pH 7.4 at 37 °C. Clearly, the PDI remained low and the decrease in polymer molecular weight was limited in the case of PLA-co-PBED 98:2H and PLA-co-PBED 98:2. In contrast, the polymer molecular weight substantially decreased in the case of the other investigated polymers, indicating considerable polyester degradation. The smaller the polymer chains, the less they are entangled and the more mobile becomes the incorporated drug. Also, the matrix porosity increases and the overall drug release properties are altered. This is not too surprising since apomorphine HCI is a fragile drug, the temperature was 37 °C, also occurred within the delivery systems. This is not too surprising since apomorphine HCI is a fragile drug, the temperature was 37 °C, also occurred within the delivery systems. This is not too surprising since apomorphine HCI is a fragile drug, the temperature was 37 °C, also occurred within the delivery systems.

### Acknowledgment

The authors are grateful for the support of this work by the French National Research Agency “ANR” (BIOSTAB and BIOPOLYCAT), the Nord-Pas de Calais Regional Council (PRIM), the “INTERREG IVA 2 Mers Sea Zeeën Cross-border Cooperation Programme 2007–2013” (IDEA), the Joint Center for Microscopy at the University of Lille (Lille, France) and OSEO.

### References

2. J.C. Middleton, A.J. Tipton, Synthetic biodegradable polymers as orthopedic de

### Table 3

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